09/888,035

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313 on May 23, 2005.

David Saliwanchik

David R. Saliwanchik, Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. SPO-115C1
Patent No. 6,861,574 B2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Atsunori Fukuda, Yoshiyuki Tanaka
Issued	:	March 1, 2005
Patent No.	:	6,861,574
For	:	Sodium/Proton Antiporter Gene

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 5, line 45:	Page 9, line 25:
"OSNHX"	--OsNHX--
Column 13, line 7:	Page 23, line 10:
"NaCl and"	-- NaCl– and--

Patent Reads:	**Application Reads:**
Column 21, line 55, (claim 8):	Claim 8, line 3 (Examiner's Amendment 6/9/04):
"comprising"	--consisting of--
Column 23, line 8, (claim 21):	Claim 25, line 4 (Amendment dated 5/19/04):
"comprising"	--consisting of--
Column 24, line 2, (claim 21):	Claim 25, line 8 (Amendment dated 5/19/04):
"comprising of the"	--comprising the--
Column 24, line 6, (claim 22):	Claim 28, line 1 (Amendment dated 5/19/04):
"A isolated"	--An isolated--

True and correct copies of pages 6-7 of the Amendment under 37 CFR §1.111 dated May 19, 2004, as well as copies of pages 9 and 23 of the application as originally filed and a copy of the Examiner's Amendment dated June 9, 2004, which support Applicants' assertion of errors on the part of the Patent Office, accompany this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,

David Saliwanchik

David R. Saliwanchik
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Attachments: Certificate of Correction;
Examiner's Amendment (6/9/04);
Copies of pages 6-7 of Amendment under 37 CFR §1.111 (5/19/04);
Copies of pages 9 and 23 of the application as originally filed.

JUN 01 2005

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,861,574 B2

DATED : March 1, 2005

INVENTORS : Atsunori Fukuda, Yoshiyuki Tanaka

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 5,
Line 45, "OSNHX" should read --OsNHX--.

Column 13,
Line 7, "NaCl and" should read --NaCl– and--.

Column 21,
Line 55, "comprising" should read --consisting of--.

Column 23,
Line 8, "comprising" should read --consisting of--.

Column 24,
Line 2, "comprising of the" should read --comprising the--.

Column 24,
Line 6, "A isolated" should read --An isolated--.

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PATENT NO. 6,861,574
No. of add'l. copies
@ 30¢ per page

FORM PTO-1050 (REV. 3-75) UNITED STATES PATENT AND TRADEMARK OFFICE

JUN 01 2005

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with David Saliwanchik on June 9, 2004.

The application has been amended as follows:

In claim 8 line 3 "consisting of" has been substituted for "comprising".

Remarks

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Cynthia Collins whose telephone number is (571) 272-0794. The examiner can normally be reached on Monday-Friday 8:45 AM -5:15 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Amy Nelson can be reached on (571) 272-0804. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

23. (Currently amended) A transformant plant ~~that it~~ that is the offspring or clone of a transformant plant comprising the transformant cell transformed with a DNA encoding a protein having an Na+/H+ antiporter activity obtained from a monocotyledonous plant selected from the group consisting of:

(a) a DNA encoding a protein comprising the amino acid sequence described in SEQ ID NO.: 2, wherein the number of amino acids that are substituted, deleted, inserted and/or added is 20 or less; and

(b) a DNA specifically hybridizing under highly stringent conditions to the DNA comprising the nucleotide sequence described in SEQ ID NO.:1, wherein highly stringent conditions comprise washing at 56 °C in a wash solution containing 0.1X SSC and 0.1% SDS~~, and~~;

wherein said ~~transformant plant~~ offspring or clone carries said DNA.

24. (Previously presented) A material for the breeding of a transformant plant comprising a transformant cell transformed with a DNA selected from the group consisting of:

(a) a DNA encoding a protein comprising the amino acid sequence described in SEQ ID NO.: 2; and

(b) a DNA comprising the coding region of the nucleotide sequence described in SEQ ID NO.: 1.

25. (Currently amended) A material for the breeding of a transformant plant comprising a transformant cell transformed with a DNA encoding a protein having an Na+/H+ antiporter activity obtained from a monocotyledonous plant selected from the group consisting of:

(a) a DNA encoding a protein consisting of the amino acid sequence described in SEQ ID NO.: 2, wherein the number of amino acids that are substituted, deleted, inserted and/or added is 20 or less; and

(b) a DNA specifically hybridizing under highly stringent conditions to the DNA comprising the nucleotide sequence described in SEQ ID NO.:1, wherein highly

stringent conditions comprise washing at 56 °C in a wash solution containing 0.1X SSC and 0.1% SDS.

26. (Canceled).

27. (Canceled).

28. (Currently amended) An isolated nucleic acid molecule having a chain length of at least 15 nucleotides that is ~~96% or more homologous~~ identical to an at least 15-nucleotide fragment of the DNA described in SEQ ID NO.: 1.

plant belonging to the *Gramineae* family;

(12) the transformant plant of (11), wherein the plant is rice;

(13) a transformant plant that is the offspring or clone of the transformant plant of any of (9) to (12);

(14) a material for the breeding of the transformant plant of any of (9) to (13);

(15) an antibody that binds to the protein of (7);

(16) a nucleic acid molecule that hybridizes with the DNA described in SEQ ID NO: 1, and which has a chain length of at least 15 nucleotides.

The present invention provides a novel Na^+/H^+ antiporter derived from monocotyledoneae, as well as a DNA encoding the same. The base sequence of the cDNA encoding the Na^+/H^+ antiporter "*OsNHX1*", derived from rice and isolated by the present inventors, is indicated in SEQ ID NO: 1. The amino acid sequence of the protein encoded by the cDNA is described in SEQ ID NO: 2.

The "*OsNHX1*" gene showed significant identity with many known amino acid sequences of the Na^+/H^+ antiporters, and especially high identity was observed at sites related to ion transport. This finding suggests that "OsNHX1"protein plays an important role in Na^+ transport in rice. It is supposed that Na^+/H^+ antiporters of plants are involved in the securement of osmotic pressure balance in the plant body under a high salinity stress. Thus, it is anticipated that the "*OsNHX*" gene especially can be applied to production of salt tolerant cultivars.

Not only "OsNHX1" protein, but also proteins with equivalent functions, are included in this invention. The term "proteins with equivalent functions to 'OsNHX1' protein" herein means that the object protein functions as an Na^+/H^+ antiporter. The activity of an Na^+/H^+ antiporter can be detected, for example, by detecting the H^+ ejection from the biomembrane

Example 4-Functional complementation experiments in yeast

Experiments for functional complementation by *OsNHX1* gene were performed using budding yeast vacuolar Na^+/H^+ antiporter gene *NHX1* mutant strain (*Δnhx1*, R100) (Nass, R. et al., (1997) The Journal of Biological Chemistry 272, 26145-26152). Budding yeast was cultured in YPD medium, SD medium, or, in the case of NaCl treatment, APG medium. *OsNHX1* gene was inserted downstream of the GAP promoter of pKT10 vector, into which *HIS3* gene was inserted. The resulting vector was introduced into budding yeast by lithium method. NaCl- and hygromycin-sensitivity of *Δnhx1* was recovered by overexpressing *OsNHX1* gene (Figure 5). Thus, it was confirmed that *OsNHX1* gene encoded a protein having vacuolar Na^+/H^+ antiporter function.

Example 5-Localization of OsNHX1 protein

A peptide synthesized on the basis of the carboxyl-terminal 16 amino acids of the amino acid sequence deduced from *OsNHX1* gene was injected into a rabbit as an antigen. Antisera obtained were purified by affinity chromatography to prepare OsNHX1 protein-specific polyclonal antibody. Antibody preparation was requested from Sawady Technology Co., LTD. By Western analyses using the OsNHX1 protein-specific antibody obtained, it was confirmed that OsNHX1 protein was largely localized in the tonoplast fraction (Figure 6). Thus, it was confirmed that OsNHX1 protein was a vacuolar Na^+/H^+ antiporter.

Industrial Applicability. According to the present invention, it is expected that isolated Na^+/H^+ antiporter gene can render salt tolerance to the plant by expressing it in the plant. Therefore, it may conduce, for example, an increase in the harvest of crops, due to improvements in salt tolerance, by transfer into useful crops such as rice, which will make them resistant to harm by salt in dry land and such.